PARATHYROID HORMONE ANTAGONISTS OR MODULATORS AND USES THEREFOR

TECHNICAL FIELD

The present invention relates to novel parathyroid hormone antagonists and methods for using such compositions. In particular, a pharmaceutical parathyroid hormone antagonist comprises a peptide having an amino acid sequence from between (SEQ ID No.2 [PTH_{2-84}]) and (SEQ ID No. 3 [PTH_{34-84}]), preferably between (SEQ ID No. 4 [PTH_{3-84}]) and (SEQ ID No. 5 [PTH_{28-84}]), or a conservatively substituted variant thereof exhibiting PTH antagonist activity and a pharmaceutical carrier or excipient. Such compositions can be used to affect the binding of parathyroid hormone to parathyroid hormone receptors, to treat a patient having hyperparathyroidism, to treat a patient having renal osteodystrophy, or to modulate *in vivo* calcium ion concentration in blood.

BACKGROUND ART

Calcium plays an indispensable role in cell permeability, the formation of bones and teeth, blood coagulation, transmission of nerve impulse, and normal muscle contraction. The concentration of calcium ions in the blood is, along with calcitriol (Vit D) and calcitonin, regulated mainly by parathyroid hormone (PTH). Extracellular calcium levels are directly affected by PTH through calcium uptake in kidney tubule cells and calcium transport to or from bone. Although calcium intake and excretion may vary, PTH serves through a feedback mechanism to maintain a steady concentration of calcium in cells and surrounding fluids. When serum calcium lowers, the parathyroid glands secrete PTH, affecting the release of stored calcium. When serum calcium increases, stored calcium release is retarded through lowered secretions of PTH.

The complete or whole form of human PTH, (hPTH), is a unique 84 amino acid peptide (SEQ ID NO. 1), as is shown in FIGURE 1. Researchers have found that this peptide has an anabolic effect on bone that involves a domain for protein kinase C activation (amino acid residues 28 to 34) as well as a domain for adenylate cyclase activation (amino acid residues 1 to 7). However, various catabolic forms of clipped or fragmented PTH peptides also are found in circulation, most likely formed by intraglandular or peripheral metabolism. For example, hPTH can be cleaved between amino acids 34 and 35 to produce a (1-34) PTH N-terminal fragment and a (35-84) PTH C-terminal fragment. Likewise, clipping can occur between either amino acids 36 and 37 or 37 and 38. Recently, a large PTH fragment referred to as "non-(1-84) PTH" has been disclosed which is clipped closer to the N-terminal end of PTH. (See R. LePage *et alia*, "*A non-(1-84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples* " Clin Chem (1998); 44: 805-810.)

The cleaved fragments of PTH vary in both biological activity and metabolic clearance rate from the circulation. For example, the N-terminal human PTH_{1-34} ($hPTH_{1-34}$) fragment has PTH agonist properties, but is rapidly removed from circulation. A daily subcutaneous administration of hPTH to patients with idiopathic osteoporosis has been shown to substantially increase their iliac trebecular bone volume. (See R. Podbesek *et alia*, "*Effects of two treatment regimes with synthetic human parathyroid hormone fragment on bone formation and the issue balance of trabecular bone in greyhounds*". Endocrinology (1983); 112: 1000-1006.)

PTH plays a role in the course of disease in a patient with chronic renal failure. Renal osteodystrophy (RO) is a complex skeletal disease comprising osteitis fibrosa cystica (caused by PTH excess), osteomalacia resulting in unmineralized bone matrix (caused by vitamin D deficiency), extraskeletal calcification/ossification (caused by abnormal calcium and phosphorus metabolism), and adynamic bone disease (contributed to by PTH suppression). Chronic renal failure patients can develop RO. Failing kidneys increase

serum phosphorus (hyperphosphoremia) and decrease 1,25-dihydroxyvitamin D (1,25-D) production by the kidney. The former results in secondary hyperparathyoridism from decreased gastrointestinal calcium absorption and osteitis fibrosa cystica from increased PTH in indirect response to an increase in serum phosphorus. The later causes hypocalcemia and osteomalacia. With the onset of secondary hyperparathyroidism, the parathyroid gland becomes less responsive to its hormonal regulators because of decreased expression of its calcium and vitamin D receptors. Serum calcium drops. RO can lead to digital gangrene, bone pain, bone fractures, and muscle weakness.

To treat secondary hyperparathyroidism, patients are given calcium and vitamin D replacement. Vitamin D analogues, such as calcitriol, stimulate intestinal calcium transport, calcium absorption in bone and calcium tubular reabsorption in kidneys. Such therapy has its dangers. Serum calcium levels must be carefully monitored. Too much dosage can induce hypercalcemia or hypercalciuria. Moreover, very serious consequences occur from calcium and phosphorus mismanagement from direct and indirect PTH suppression therapy. Soft tissue calcification results in a five to fifteen times higher incidence of myocardial infarction among end stage renal dialysis patients as compared to age matched diabetes patients. The secondarily hyperplastic parathyroid glands escape PTH control over calcium, a condition referred to as tertiary hyperparathyroidism.

Another treatment proposed for patients with excess PTH is to administer parathyroid hormone analogues which inhibit the biological activity of PTH. U.S. 5,093,233 and U.S. 4,968,669 disclose N-terminal PTH analogues (PTH_{7-34} and PTH_{8-34}) having substitutions at the 8, 12, 18, and/or 34 amino acid positions. These analogs bind to PTH cell surface receptors but do not stimulate a change in the second messenger concentration, *i.e.*, act as a hormone for calcium ion concentration. PTH activity can also be inhibited by unsubstituted PTH fragments, namely PTH_{3-34} or PTH_{7-34}, however, these fragments are so weak in their antagonist properties that they do not have practical or beneficial significance.

DISCLOSURE OF THE INVENTION

The present invention relates to novel PTH antagonists and methods for using such compositions. In particular, a pharmaceutical PTH antagonist comprises a peptide having an amino acid sequence from between (SEQ ID No.2 [PTH_{2-84}]) and (SEQ ID No. 3 [PTH_{34-84}]), preferably between (SEQ ID No. 4 [PTH_{3-84}]) and (SEQ ID No. 5 [PTH_{28-84}]), or a conservatively substituted variant thereof exhibiting PTH antagonist activity and a pharmaceutical carrier or excipient. By truncating the N-terminal end of PTH, the adenylate cyclase activity is substantially eliminated. By having a PTH fragment extending at least from the 34th amino acid to the C-terminal end, one not only retains the binding capacity such that the peptide has antagonist properties greater than PTH_{3-34}, but one also retains the ability of the body to use natural clearing mechanisms for removing the PTH antagonist.

The instant compositions can be used in a number of ways. First, one can affect the binding of parathyroid hormone to parathyroid hormone receptors. By adding the present compositions to a medium in contact with PTH receptors, the receptors can be tied up with respect to PTH or PTH analogs in that the PTH binding site is blocked by the proposed antagonist administration. Second, one can use the present compositions to treat a patient having hyperparathyroidism. By adding the present compositions in a therapeutically effective, but non-toxic amount, one can counter the hypercalcemia caused by increased PTH secretion, and thus, the increased binding and activation of PTH receptors. Third, one can treat a patient having renal osteodystrophy by adding the present compositions in a therapeutically effective, but non-toxic amount, one can reduce the development of osteitis fibrosa cystica caused by PTH excess. Fourth, one can modulate *in vivo* calcium ion concentration in blood. By adding the present compositions in a therapeutically effective, but non-toxic amount, one can decrease serum calcium because while serum PTH levels increase in response to a serum phosphorus increase, the binding of PTH antagonist to PTH receptors prevents the increased PTH level from increasing gastrointestinal absorption of calcium as well as affect calcium stored in bone.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a diagrammatic view of hPTH.

FIGURE 2 is a graph showing the change in serum calcium using PTH alone, the present PTH antagonist alone, a combination of PTH and present PTH antagonist, and a control.

BEST MODES FOR CARRYING OUT THE INVENTION

In disclosing the present invention, one should remember that there are a number of closely analogous, species dependent forms of PTH. The amino acid sequence of hPTH is shown in FIGURE 1. However, for rat PTH, bovine PTH, or porcine PTH, for example, one finds the substitutions at some of the amino acids in the hPTH sequence. For the purposes of the present invention, one can use interchangeably truncated forms of these PTH's, although it is preferred to use a PTH having a sequence matching the species in which the PTH antagonist is used.

Preferred PTH antagonists of the present invention have an amino acid sequence from between $PTH_{2\text{-}84}$ and $PTH_{28\text{-}84}$ or a conservatively substituted variant thereof exhibiting PTH antagonist activity, with the most preferred form being from between $PTH_{2\text{-}84}$ and $PTH_{9\text{-}84}$.

PTH antagonist peptide preparation

In order to make the present compositions, one can use any conventionally known method. For example, one can use recombinant DNA methods to produce the desired compound.

Alternatively, one can use an automated peptide synthesizer, such as Model 431 made by Applied Biosystems, Inc. (Foster City, California, U.S.A.) Fmoc (9-fluoronylmethoxycarbonyl) can be used as the alpha-amino protecting group. All amino acids and solvents are available from Applied Biosystems and are of synthesis grade. Following synthesis, the peptide is cleaved from the resin, and side chains are de-blocked, using a cleavage cocktail containing 6.67% phenol, 4.4% (v/v) thioanisole and 8.8% ethanedithiol in trifluoroacetic acid (TFA). The cleaved peptide is precipitated and washed several times in cold diethyl ether. It is then dissolved in water and lyophilized. The crude peptide is subjected to amino acid analysis (Waters PICO-TAG System, Boston, Massachusettts, U.S.A.) and reversed-phase HPLC using a VYDAC (TM) C8 column with 0.1% TFA in water and 99.9% acetonitrile in 0.1% TFA as the mobile buffers. The presence of a single major peak along with the appropriate amino acid composition is taken as evidence that the peptide is suitable for further use.

PTH Pharmaceutical compositions

The present PTH antagonist peptides exhibit both oral and parenteral activity and can be formulated in solid or liquid dosage forms for oral, parenteral, intranasal, topical, or injectable administration using known carriers, excipients, or the like. The exact amount of present PTH antagonist used can vary depending upon the degree of antagonist property desired, the route of administration, or the duration of the treatment, as is known to the art.

Antagonist Properties

The present PTH antagonists have the ability to reduce the increase in serum calcium normally caused by PTH or a PTH agonist analog. FIGURE 2 is a graph demonstrating such a property. Twenty five rats were used in a demonstration of the effect

of the present PTH antagonists. All of the rats had their parathyroid glands removed. Five rats received an *i.v.* injection of a saline control. The serum calcium of the control rats was measured and on average was lowered over time by about 0.18 mg/dl, as a result of the parathyroidectomy. Nine rats received an *i.v.* injection (10 μg/kg) of hPTH obtained from Bachem, AG of Bubendorf, Switzerland. The serum calcium of the hPTH rats was measured and on average was raised by about 0.65 mg/dl. Five rats received an equimolar *i.v.* injection of a PTH_{7-84} (the PTH antagonist) also obtained from Bachem, AG of Bubendorf, Switzerland. The serum calcium of the PTH antagonist rats was measured and on average was lowered over time by about 0.30 mg/dl. Finally, six rats received an *i.v.* injection comprised of hPTH (10 μg/kg) and an equimolar amount of PTH_{7-84}. The serum calcium of the hPTH/PTH antagonist rats was measured and on average remained substantially the same, raising only about 0.03 mg/dl. Thus, the present composition was able to prevent the substantial serum calcium increase normally associated with an administration of hPTH to rats having hypoparathyroidism, and quite unexpectedly, is much more potent in its antagonist property than the reported antagonist PTH_{3-34}.

The ordinarily skilled artisan can appreciate that the present invention can incorporate any number of the preferred features described above.

All publications or unpublished patent applications mentioned herein are hereby incorporated by reference thereto.

Other embodiments of the present invention are not presented here which are obvious to those of ordinary skill in the art, now or during the term of any patent issuing from this patent specification, and thus, are within the spirit and scope of the present invention.